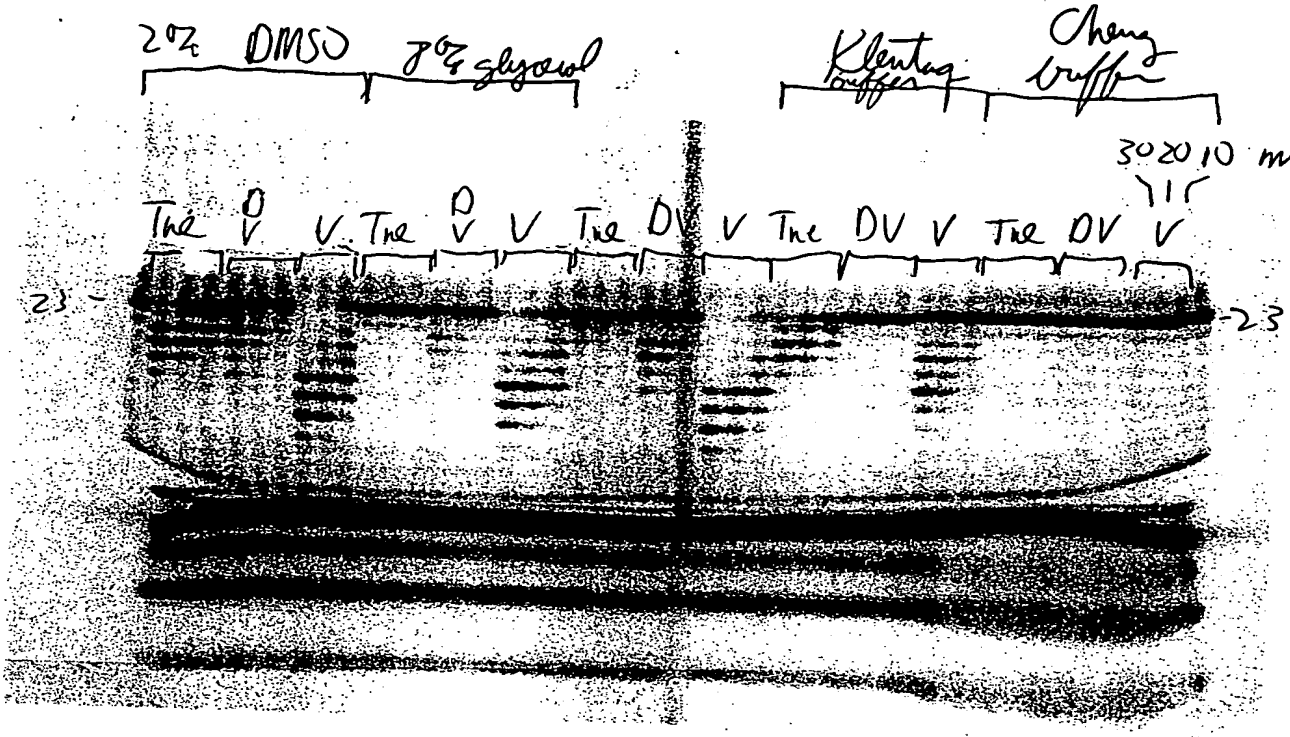
82

Project No._____
Book No._____ TITLE _____

From Page No.__



Result.

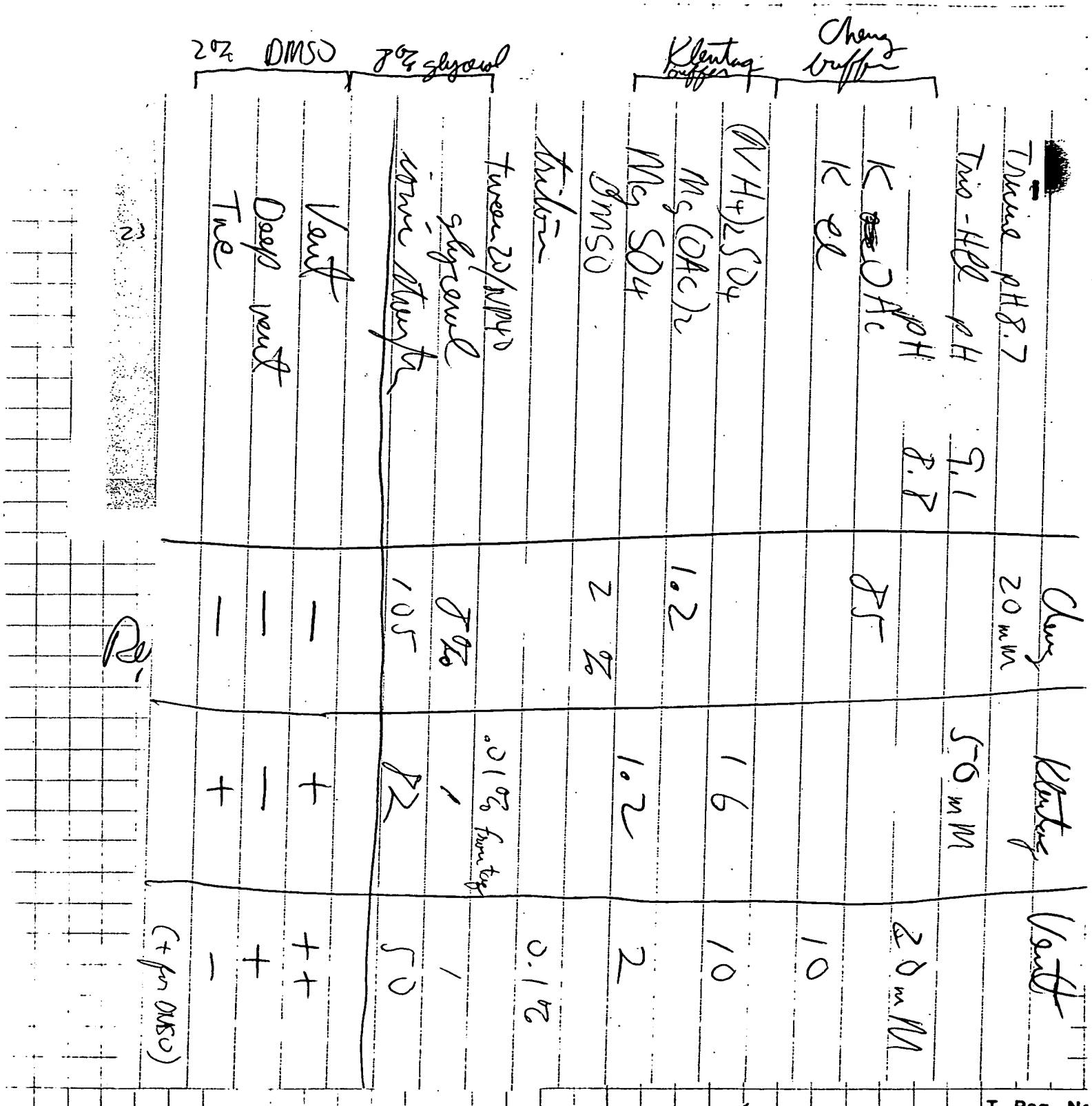
Witnessed & Understood by me,
Deanna Polan

Date
11/29/94

Invented by
Recorded by

Date
11-5-94

T Page N.



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Date
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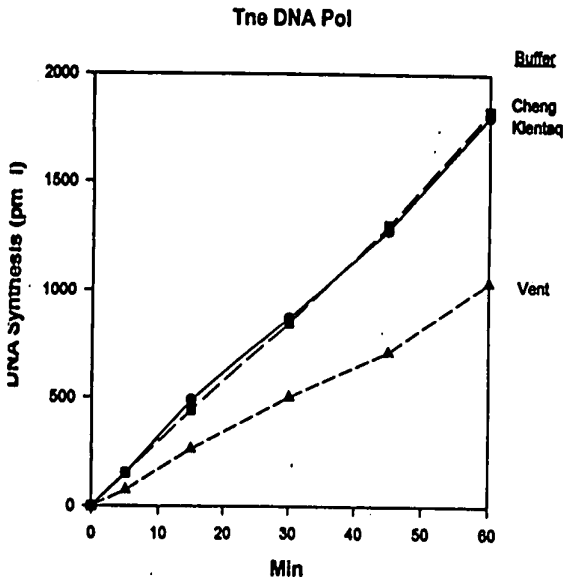
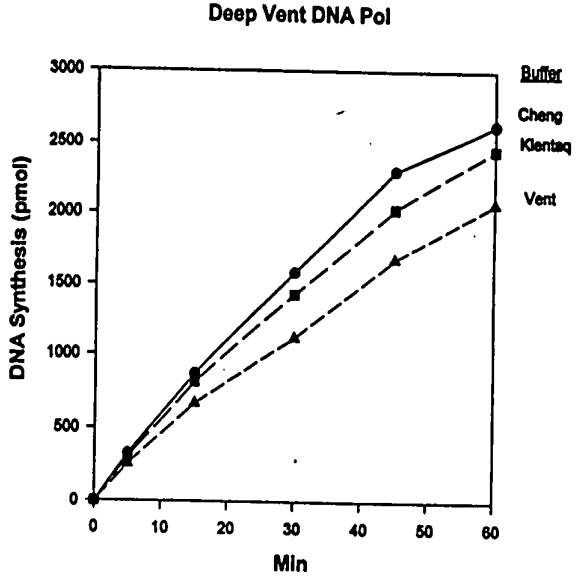
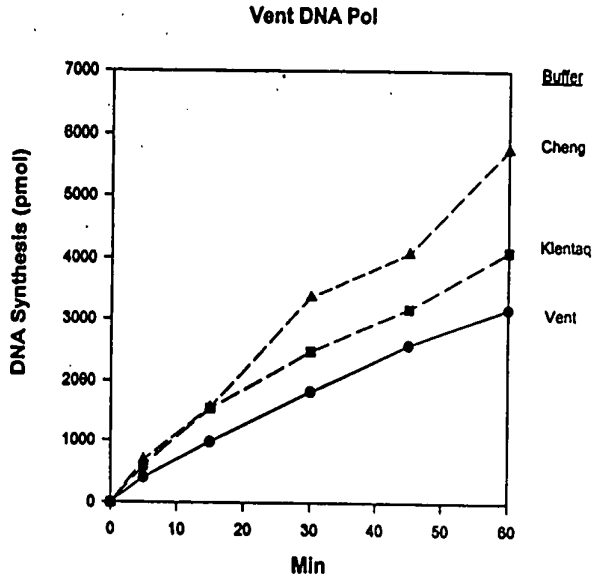
Date
11-5-94

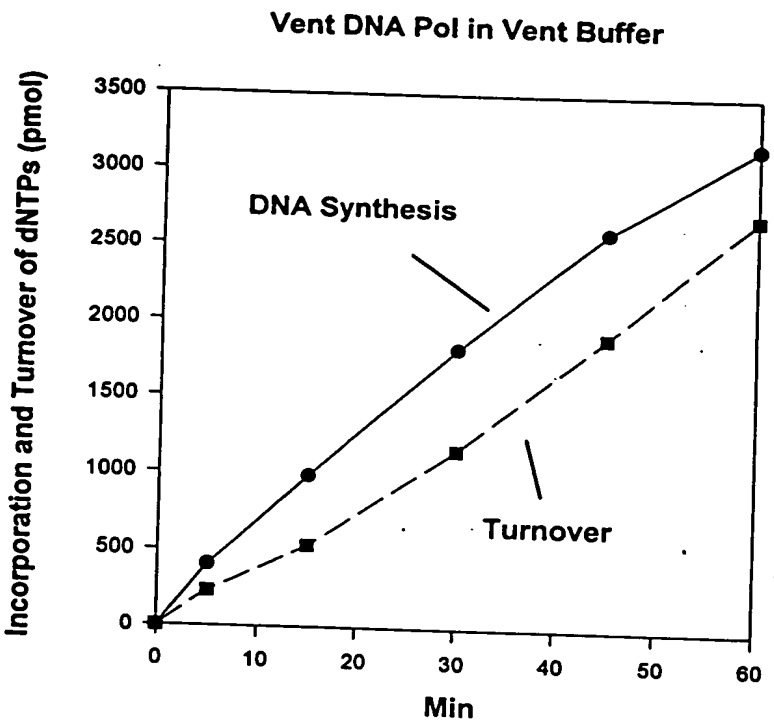




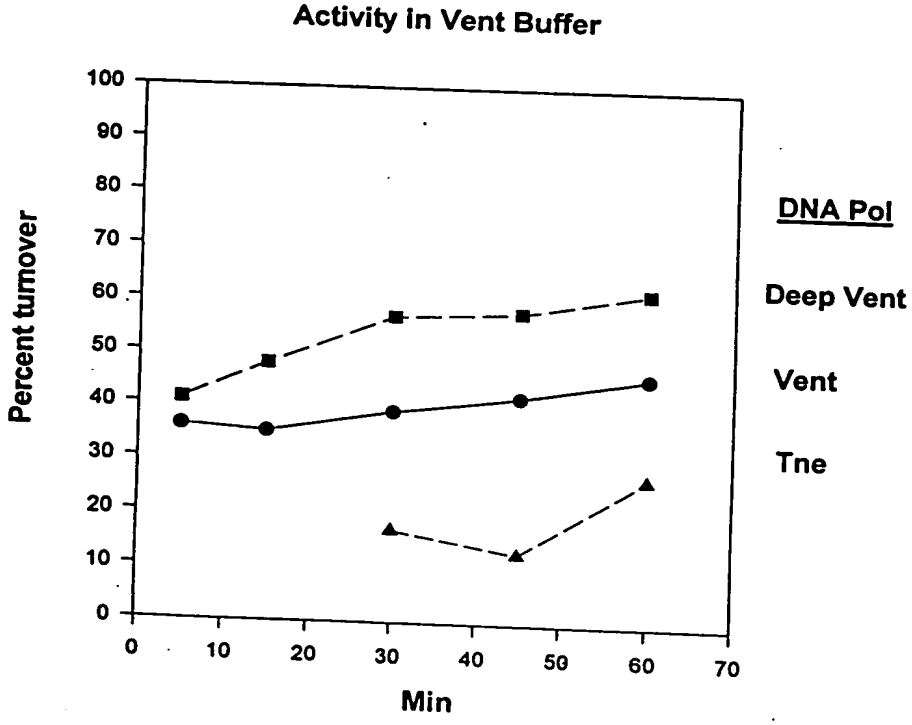


In each case, DNA synthesis is lower in
Primer degradation was highest in Vent

got Turnover
by DNA synthesis
↓ below

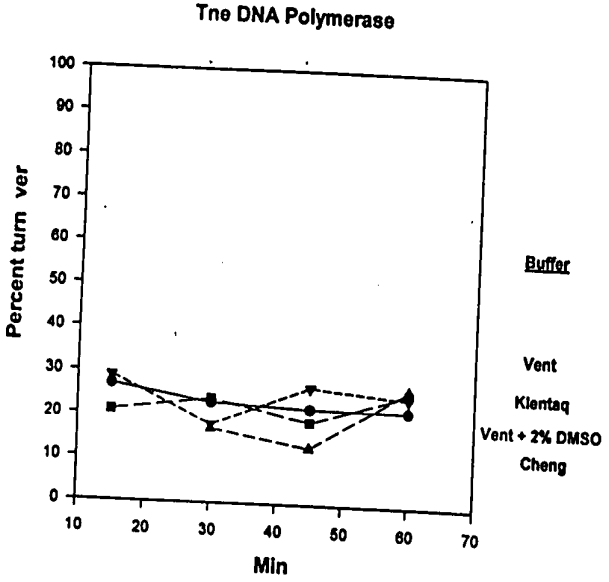


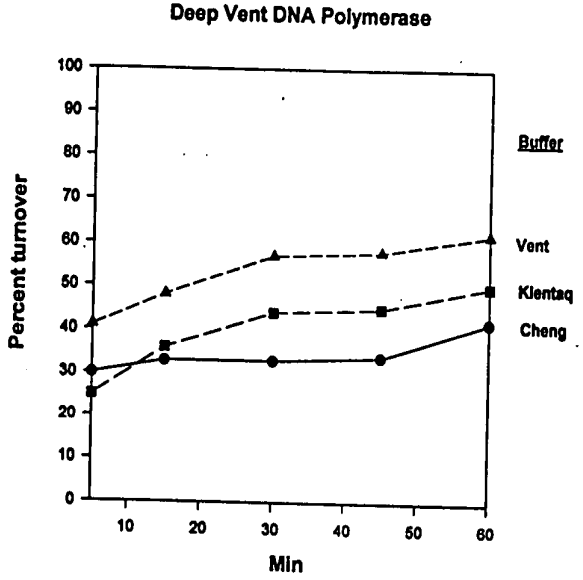
DNA synthesis and turnover to dNMP

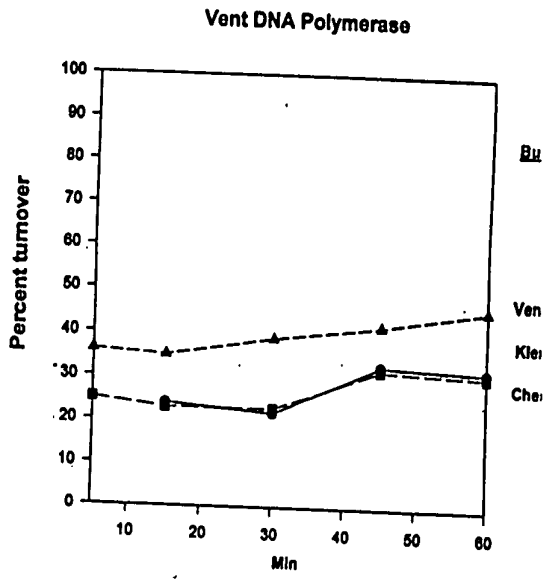


Percent turnover = $\frac{\text{turnover}}{\text{incorporation + turnover}}$

Deep Vent has higher turnover than Vent as expected. Tne is ~2x lower than Vent and Deep Vent







Effect of buffer on turnover is not large compared to effect on primer degradation

Project No.______
Book No.______
TITLE Turnover for Vent, deep Vent (follow p. 61, 7)

From Page No.__

	(A)	(B)	(C)
H_2O	399 ~~400~~	~~481~~ 464	~~489~~ 47
5x Cheng buffer	133		
10x Klentaq		66.7	
10x Vent buffer			66.7
Taq storage buffer	6.7λ		
3.7 mg/ml activated DNA	90		
dA,T,G-T-TP 10mM each	3.33		
^{32}P αdATP 10mCi/ml	1.2λ		
$Mg(OAc)_2$ 50 mM	16 μl		
$MgSO_4$ 100mM		8μl	
DMSO 100%			
	0.65 ml	0.633	0.633, 0.650 use 1

	1	2	3	4	5	6	7	8
	195	195	195	190	190	190	190	190
* Taq storage buffer				4	4	–		
Vent .08u/λ	4			4			4	
Deep Vent .08u/λ		4			4			4
Taq 0.08u/λ			4			4		

H_2O 1 2 3 6 6 6

prewarm to 70°C, start by addition of pol

remove 15μl to 5μl 0.2M EDTA → spot 15μl on G... and remove 5μl to 5μl Kill solution (20μmol/ml dA... 100 mM EDTA) at:

0, 5, 15, 30, 45, 60 min

spot 2μl on PEI, resolve in 1M LiCl

* dilutions of pols same as p.81

Results: see graph on p.81

T Pag

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ag N . (1)

14.4 ✓
✓
✓
✓

66.7 20
→ 27 ✓ (0.1 µl / 100 µl PCR ⇒ Cf = 0.005% Tween 20/NP40
this makes up for no TFL here - its present in Joes long PCR Rxn.

→ 1 ✓ (Cf = 50 µM each)

→ 0.36 ✓ (~20 x 10^6 total cpm)

✓ (1.2 mM MgOAc)

✓ (1.2 mM MgSO4 in Klentaq buffer
2 mM MgSO4 in 1x Vent buffer)

4 µl ✓ Cf = (2% DMSO)

10
19.4 ✓ (0.4 units total/each pol)

4

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[redacted]	11/29/94	Recorded by	11-9-94

Turnover

Project No. 06

Book No. Turnover **TITLE** Incorp pmol

min	No.	Value				
0	1	543.00	BKGD			
5	2	650.00	110	(14)		From p
15	3	1014.00	486	24		
30	4	1485.00	971	22	V	Cheng
45	5	2627.00	2148	34		
60	6	3187.00	2725	32		
	7	525.00	BKGD			
	8	662.00	141	30		
	9	948.00	436	33	DV	
	10	1271.00	769	33		
	11	1677.00	1188	34		
	12	2340.00	1871	42		
	13	624.00	BKGD			
	14	694.00	72	(32)		
	15	796.00	177	27	TNE	
	16	880.00	264	23		
	17	976.00	363	22		
	18	1110.00	501	22		
	19	805.00	BKGD	785 Ave		Bentley
	20	977.00	192	25		
	21	1409.00	467	23	V	
	22	1803.00	762	23		
	23	2832.00	1533	32		
	24	3299.00	1883	31		
	25	774.00	BKGD			
	26	918.00	99	25		
	27	1406.00	415	36	DV	
	28	2277.00	1118	44		
	29	2989.00	1651	45		
	30	4085.00	2472	50		
	31	777.00	BKGD			
	32	813.00	21	(12)		
	33	947.00	121	21	TNE	
	34	1136.00	263	24		
	35	1204.00	314	19		
	36	1631.00	633	26		
	37	919.00	BKGD	922 ave		
	38	1284.00	251	36		
	39	1754.00	530	35		
	40	2728.00	1150	39	V	
	41	3910.00	1903	42		
	42	5168.00	2704	46		
	43	924.00	BKGD			
	44	1205.00	180	41		
	45	1892.00	617	47	DV	
	46	3234.00	1472	57		
	47	4572.00	2325	58		
	48	6365.00	3467	62		
	49	863.00	BKGD			
	50	901.00	—			
	51	953.00	20	(7)	TNE	
	52	1083.00	103	17		
	53	1085.00	103	13		
	54	1529.00	386	27		
	55	984.00	BKGD			
	56	891.00	—			
	57	1067.00	92	29	TNE	
	58	1086.00	104	18		
	59	1336.00	264	27		
	60	1467.00	347	25		

No.		Value	pmol
61		269.00	
62		7412.00	679
63		16953.00	1553
64	V	36825.00	3374
65		44610.00	4087
66		62771.00	5752
67		241.00	
68		3518.00	322
69		9506.00	871
70	DV	17320.00	1587
71		25050.00	2296
72		28643.00	2625
73		324.00	
74		1974.00	151
75		5340.00	489
76	TNE	9478.00	869
77		13880.00	1272
78		19753.00	1810
79		321.00	
80		8826.00	588
81		23029.00	1533
82	V	37324.00	2485
83		47661.00	3173
84		61758.00	4112
85		404.00	
86		4493.00	299
87	DV	12238.00	815
88		21497.00	1431
89		30491.00	2030
90		36800.00	2450
91		214.00	
92		2257.00	150
93	TNE	6671.00	444
94		12685.00	845
95		19429.00	1294
96		27534.00	1833
97		239.00	
98		7128.00	404
99	V	17335.00	981
100		32171.00	1821
101		45795.00	2592
102		56065.00	3174
103		318.00	
104		4474.00	253
105	DV	11839.00	670
106		19756.00	1119
107		29674.00	1680
108		36540.00	2069
109		261.00	
110		1566.00	74
111		4647.00	265
112	TNE	8879.00	503
113		12496.00	707
114		18327.00	1037
115		295.00	
116		1709.00	80
117		4261.00	214
118	TNE	8343.00	[illegible]
119		12504.00	708
120		18443.00	1048

Page 49

Witnessed by me Deborah Polans **Date** 11/29/94

Invented by [signature] **Recorded by** 11-9-94

ıg N .

dAMP BKGD:
1. Cheng mix = 564 ave
2. Kleuting mix = 785
3. Vent mix = 922

spot
Cheng 75821 cpm $\left(\frac{50\mu l \text{ Rxn vol}}{2\lambda \text{ spotted}}\right)\left(\frac{200}{195}\right)\left(\frac{1}{2500 \text{ pmol dATP}}\right)\left(\frac{1}{4}\right)$ = 194 cpm/pmol ut

Kleuting 267 cpm/pmol

Vent 314 cpm/pmol

pmol incorp (200 μl Rxn) =

$$\frac{\text{cpm}}{\text{cpm/pmol}}\left(\frac{200}{15\lambda}\right)\left(\frac{20}{15}\right)$$

pmol turnover (200 μl Rxn) =

$$\frac{\text{cpm} - \text{BKGD}}{\text{cpm/pmol}}\left(\frac{200}{5}\right)\left(\frac{10}{2}\right)$$

$$\%\text{ turnover} = \frac{\text{pmol turnover}}{\text{pmol turnover} + \text{pmol incorp}}$$

121 75821.00
122 104512.00

T Page No.

d & Understood by me,	Dat	Invented by	Date
[signature] Polarp	11/29/94	[signature]	11-10-94
		Rec rd d by	

PAGES 88-89 OF NOTEBOOK WERE BLANK

assay carried Project No.______
90 unit at Frederick. Book No.______ TITLE Repeat unit assay QC for rTaq lot # EKBT1 done on P. 61 except Amplitaq lot # 9959 for control

From Page No.__

lot EKBT1 is ~ 4.01 u/μl based on P. 61

1. starting dilutions of EKBT1:

	1:80 (estimate cf = 5 u/λ)	1:160 (estimate cf = 2.5u)
lot EKBT1	5 μl	5 μl
Taq storage buffer	395 μl	795 μl
	actual is 4.03 u/λ	actual is 2.01 u/
	Vf = 400 μl	Vf = 800 μl

2. 1/600 dilutions

serial dilution #	1-6	7-12	13-18	19-24	25-30	31-36	37-42	43-48	49-54
	I	II	III	IV	V	VI	A-1	A-2	A-3
1:80 dil	3λ	3	3						
1:160 dil				3	3	3			
Amplitaq 5 u/λ lot #							3	3	3
dilution buffer	1797 μl →								

Vf = ~~2000~~ 1800 μl

Vortex 5A use from b 20 and 40 mi

dilute I - A-3 as shown for I below:

3. Serial dilutions

		dilution buffer	
serial dilution #	1	100λ	↑300λ
	2	100λ	↑300λ
	3	100λ	↑300λ
	4	100λ	↑300λ
	5	100λ	↑300λ
	6	1 ml of I	

dilute I - III and assay
then dilute IV - VI and assay
then dilute A-1 - A-3 and assay

SA I-III = 45 μl assay mix + 5 μl dil buffer, do same for IV-VI
spot 4X 5 μl ~~on GFC~~ in 4 ml aquasol
Blank is 45 μl assay mix + 5 μl dil buffer → spot on GFC along with other

To Page No.

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[signature]	1/6/95	[signature]	11-15-94
		Recorded by	

age N .

55-57 = Blank for I-III, IV-VI and A-1 - A-3 respectively

, 58-61 = SA for I-III

62-65 = SA for IV-VI

Result:

using amplitag Lot #9957 here gives an unit value of ~~320.0 u/ml~~ 323.4 u/ml compared to 401 u/ml (found on P.61, 10-1-94)

To Page No.

ssed & Understood by m ,	Date	Inv nted by	Date
	1/6/95	Recorded by	11-15-94

Project No.________

Book No.________ TITLE New dilution of EKBT1 to 10u/λ for Larry Morty

From Page No.__

* will use old unit value of 401 u/λ so can ma[...] old dilution ~~&~~ Jerry used in October (see P91 where final unit determination for EKBT1 is 32~~3~~.4 units/μl.

Calibrated P20 (P0[...] its exactly 10 μg fo[...] for P1000 (P0187) use[...] 378 μl which ga[...] 391 μg

Tag storage buffer	391 μl
Tag lot # EKBT1 ("401" u/μl) * see above	10 μl
	Vf = 401 μl (10 units/μl)

1. bring Tag storage buffer to room Temp.
2. bring small ~~aliquot~~ aliquot of EKBT1 (main stock) to room temp.
3. deliver 10 μl Tag into 378 μl storage buffer, rinse ~10 times (ie. triturate)
4. mix with P1000 to get in all storage buffer
5. vortex 5 sec
6. mix end over end in cold room 2 hr.

T Pag N[...]

Witn ssed & Und rsto d by me, Deearna Bolamp Dat 1/6/95

Invented by [signature] Dat 11-30-94

R c rded by